Shigeya NIIZEKI**, Makoto NISHIDA*** & Siro KURITA***:

Cytotaxonomy of Ophioglossales I*
Chromosome numbers of *Japanobotrychium* in Japan

新関滋也**・ 西田 誠***・ 栗田子郎***: ハナヤスリ目の細胞分類学 I* ナツノハナワラビ属の染色体数

(Pl. XII & XIII)

According to the characters of vascular bundle systems in the phyllomophores and of morphology of embryos, the genus *Botrychium* was usually divided into three subgenera, i. e., *Sceptridium*, *Eubotrychium* *Osmundopteris*. Nishida (1952), however, elevated this genus to a family Botrychiaceae and also did these three subgenera to generic stata, changing the generic-name of *Osmundopteris* into *Japanobotrychium* (Nishida ex Tagawa, 1959). In the family Botrychiaceae, many cytological studies have been carried out by various authors until present (Okuno, 1936; Manton, 1950; Britton, 1953; Manton and Sledge, 1954; Wagner, 1955; Gopal-Iyengar, 1956; Ninan, 1958; Verma and Loyal 1960). From the data of these studies, it is revealed that almost all species of Botrychiaceae may have the gametic chromosome number of either 45 or its multiples, with an exception of *Japanobotrychium virginianum* (L.) Nishida from N. America having n = 92. In Japan, there are two species of the genus *Japanobotrychium: J. virginianum* (L.) Nishida and *J. strictum* (Und.) Nishida, and the meiotic behaviors in these ferns will be described in this paper.

Materials and Methods

The plants used in the present study were collected at some localities in Chiba Prefecture and were transplanted in our laboratory. Young fertile fronds were fixed in 1:3 acetic-alcohol mixture for about 24 hours, then they were squashed and stained in aceto-carmine. The text figures were prepared from the photographs by inking in and bleaching them.

Observations and Discussions

Japanobotrychium virginianum (L.) Nishida. Gametic chromosome number

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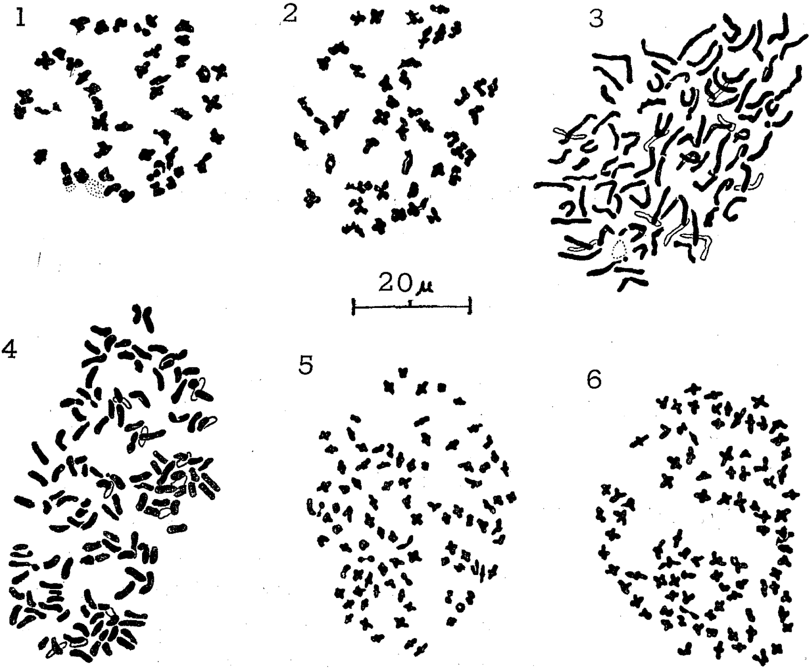

Fig. 1—6. Chromosomes of *Japanobotrichium*. 1, 2. *J. strictum*, n=44-45. 3. A normal archesporial cell of *J. strictum*, (ca 90 chromosomes). 4. A triploid archesporial cell of *J. strictum*, (ca 135 chromosomes). 5, 6. *J. virginianum*, n=97.

were counted at the first meiotic metaphase and late diakinesis of spore mother cells (Figs. 5, 6 and Pl XIII, 4), and we found 92 bivalent chromosomes. But these division figures always contained one to three small, loosely paired and dumbbell shaped bivalents. In *J. lanuginosum* (Wall.) Nishida, three to four chromosomes of this kind were observed (see Manton and Sledge, 1954), whereas *B. lunaria* (L.) Sw. (*Eubotrychium*) does not have such chromosomes (see Manton, 1950).

The first cytological studies of the present species were made by Britton (1953), and he counted approximately 92 bivalents at the first meiotic metaphase. Wagner (1955) ascertained that the haploid chromosome number of this species is exactly 92, and concluded that the present species would be a tetraploid derived from monoploid having a number of n = 46. Gopal-Iyengar (1956), however, obtained clear count of n = 90 in the materials from Bombay. Moreover, Manton and Sledge (1954), Ninan (1958) and Verma and Loyal (1960) also clearly demonstrated the number of chromosomes as n = 90 in the samples of *J. lanuginosum* (Wall.) Nishida from Ceylon, South India and Himalayas. So far, no plants having a gametic chromosome number of 46 have been reported, though the existence of such was suggested by Wagner (1955).

Considering the data mentioned above, we assume that there are two cytologically different races in *Japanobotrychium.* The race possessing a gametic chromosome number of 92 may be a derivative from a race possessing n = 90, because the gametic chromosome number of 46 has never been reported even among the Botrychiaceae, and also the gametic chromosome number of 90 has rather frequently reported in this genus. So, against the opinion of Wagner (1955), we can conclude that the present species from America and Japan may not be a simple product of tetraploidization of x = 46.

Japanobotrychium strictum (Und.) Nishida. At late diakinesis and metaphase of the first meiotic division, 44—45 bivalents were observed (Fig. 1, 2 and Pl. XII, 1). Each of bivalents is very diversified in size. Sometimes one to two loosely paired dumbbell shaped bivalents, which were also found in *J. virginianum*, were observed. Separation of bivalents at the first anaphase are regular (Pl. XII, 2), and the normal tetrads are formed. In some archesporial cells, the somatic chromosome number was counted as 2n = ca 90. Exceptionally, however, a few archesporial cells have approximately 135 chromosomes as shown in Pl. XIII, 3 and in Fig. 4. Almost all of chromosomes in this triploid archesporial cell have terminal centromeres, but some of them, less than 12, have

Tab. I. Chromosome number of *Japanobotrychium.*

Species	Locality	Chromosome number(n)	Investigators
J. lanuginosum	Ceylon	90	Manton & Sledge, 1954.
	South India	90	Ninan, 1958.
	Himalayas	90	Verma & Loyal, 1960.
J. virginianum	N. America	91—92	Britton, 1953.
	N. America	92	Wagner, 1955.
	Bombay	90	Gopal-Iyengar, 1956.
	Japan	92	Niizeki, Nishida & Kurita.
J. strictum	Japan	44—45	Niizeki, Nishida & Kurita.

sub- or median centromeres. Although the processes of the production of such triploid archesporial cells have not been explained, their occurrence seems to be of deep significance. We have found a hexaploid species in the genus *Sceptridium: S. japonicum* (Prantl.) Lyon*. And it is believed that, in the usual leptosporangiate ferns having terrestrial gametophytes, the hexaploid species may be originated in hybridization. In the case of Ophioglossales, however, it seems to be impossible to form hybrids between any species, because they produce the subterranean gametophytes. Accordingly, it is natural to consider that the hexaploid species, *S. japonicum* (n = 135), might be induced through the mechanism which is analogous with that for the induction of the triploid archesporial cells in *J. strictum*. As shown in Tab. 1, the gametic chromosome number of 44-45 have never been reported in this genus by this time, it thus appears that *J. strictum* is the first example.

Summary

Two species of the genus *Japanobotrychium* were studied cytologically. *J. virginianum* has n = 92 as already reported by Britton (1953) and Wagner (1955), and *J. strictum* has n = 44—45, which is the first example of diploid in this genus. Exceptionally a few archesporial cells of *J. strictum* have approximately 135 chromosomes.

References

1) Britton, D.M., Amer. Journ. Bot., **40:** 575-583 (1953). 2) Gopal-Iyengar, Proc. Ind. Soc. Cong. (1956), Cited from Ninan (1958). 3) Manton, I., Problems of cytology and evolution in the Pteridophytes, Camb. Univ. Press. (1959). 4) Manton, I. and Sledge, W.A., Phil. Trans. R. Soc. London, Ser. **238**B: 127-185 (1954). 5) Nishida, M., Journ. Jap. Bot., **27:** 271-278, 165-171 (1952). 6) ———, Ame. Fern Journ., **50:** 127-133 (1960). 7) Ninan, C. A., Cytologia, **23:** 291-316 (1958). 8) Okuno, S., Bot. Mag. Tokyo, **50:** 332-337 (1936). 9) Tagawa, M., Journ. Jap. Bot., **33:** 199-204 (1959). 10) Verma, S. C. and Loyal, D. S., Curr. Sci., **29:** 69-70 (1960). 11) Wagner, W. H. Jr., Rhodora, **57:** 219-240 (1955).

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ナツノハナワラビとナガホノナツノハナワラビの染色体数を調べた結果，前者は n=

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* Cytotaxonomy of Ophioglossales II. Chromosome numbers of *Sceptridium* (in press).

92であり後者はn=ca 45であった。Britton(1953)およびWagner (1955) もアメリカ産のナツノハナワラビで同じくn=92を報告している。とくにWagnerは，この種は46の基本染色体数を持つものより由来した4倍体であろうと論じている。しかしGopal-Iyengar (1956) がBombay産のナツノハナワラビでn=90を報告しており，またn=46の染色体数を持つ種はまったく知られていないことから，我々はn=92のナツノハナワラビはn=90の系統から派生したものだろうと考える。一方，ナガホノナツノハナワラビにはまれに3倍性の(3n=ca 135)胞原細胞が形成されることがわかったが，この事実はオオハナワラビに見られる6倍体(n=135)の起源について一つの示唆を与える。

○日本産苔類2種の記録について（井上 浩） Hiroshi INOUE: Notes on two hepatics recorded from Japan

日本産の苔類については最近ようやくその実体が明らかになりつつあり，いくつかの科についてはモノグラフも発表された。しかし，日本から記録されたいくつかの種類の中にはその記録のもとになった標本が入手できないなどの理由から，記録の正否を再検討することができないものが多い。最近，国立科学博物館のコケ類の標本を整理中にこのような古い記録のもとになった標本がいくつかみつかったので，このうちの2種について検討した結果を公表しておきたい。

Cephalozia lacinulata (Jack.) Spruce 牧野富太郎先生によって安房清澄山から記録され (植雑 **11**: 35. 1897), Stephani の同定によっている。Stephani はおそらくこの牧野先生の資料をもとにしたと思われるが, "Hepaticae Japonicae" (Bull. Herb. Boiss. **5**: 76～108. 1897) 中に本種を記録したが, Species Hepaticarum **3** (1908) には "Hab. Germania prope Salem (Jack.)" とだけあって日本は記録していない。尼川氏 (服部植研報 **8**: 61. 1952) も本種にふれ, "我国フロラより除外すべきであろう" としている。牧野先生の標本は Stephani に送付した一半と思われるが, "安房清澄山。明治29年4月6日。牧野富太郎" とあり, "*Cephalozia lacinulata*—det. Stephani" とされている。標本は *Calypogeia trichomanes* や蘚類が多数混生しているが, *Cephalozia bicuspidata* subsp. *otaruensis* が含まれているので, このものの誤認であると考えられる。ステリルの時には *C. lacinulata* と *C. bicuspidata* subsp. *otaruensis* のある形 (var. *setiloba* など) は非常に似てくる。

Plagiochila chinensis Steph. 本種は Stephani によって記録された (Species Hepaticarum **2**: 116. 1903) が, 私はこれについて *P. ovalifolia* var. *miyoshiana* の誤りであろうとしておいた (服部植研報 **20**: 103. 1958)。記録のもとになったものは "Okudaira no. 14" で, 伊予で採られたものであるが, 調べた結果はやはり *P. ovalifolia* var. *miyoshiana* であった。(国立科学博物館)

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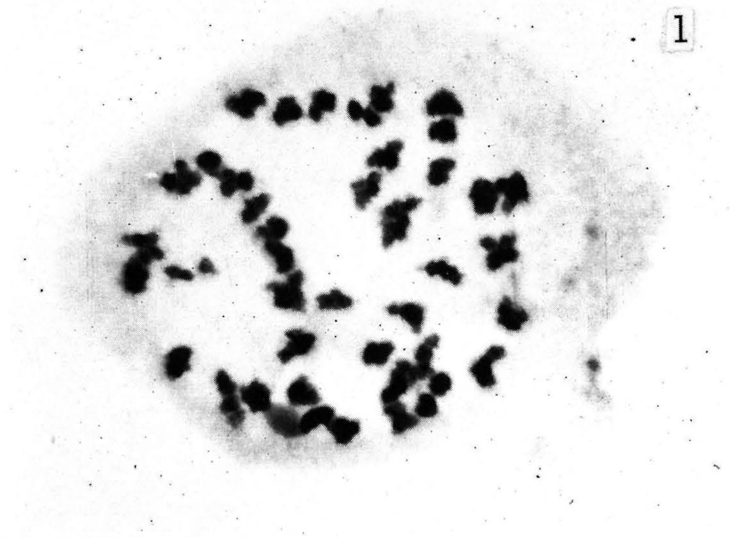

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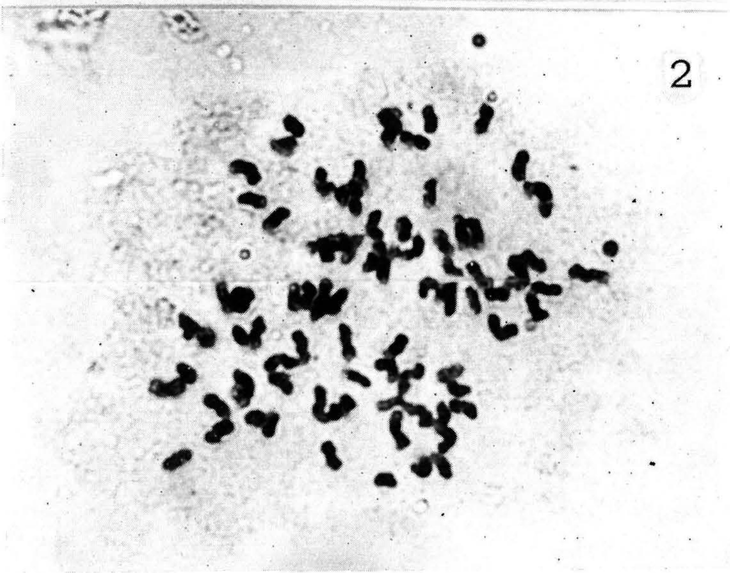

1. Metaphase of the first meiotic division of *J. strictum*, showing 44-45 bivalent chromosomes. 2. Anaphase of the first meiotic division of *J. strictum*.

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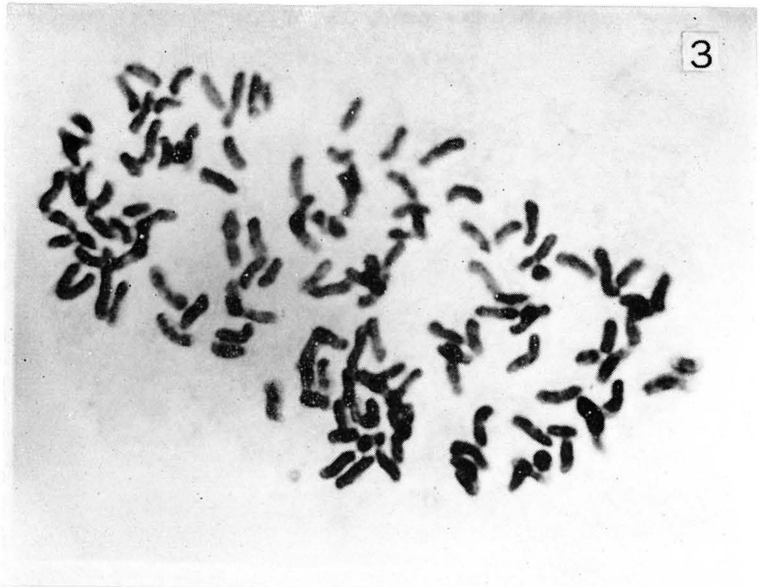

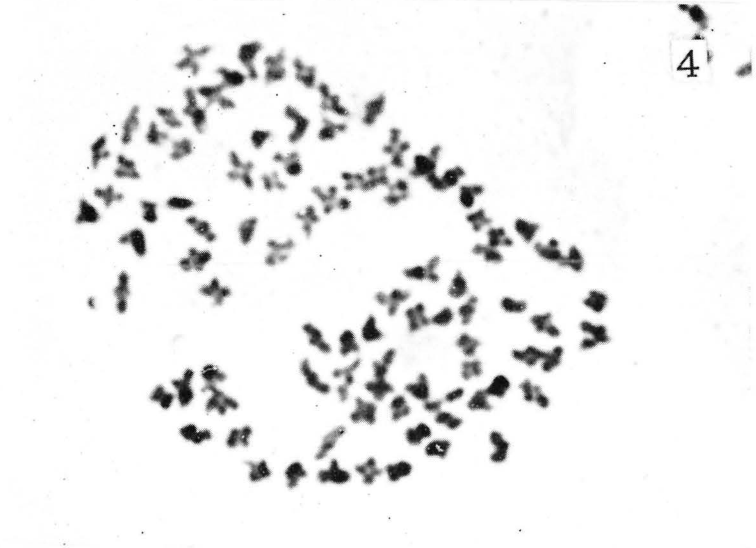

3. A triploid archesporial cell of *J. strictum*, having approximately 135 chromosomes. 4. Late diakinesis of *J. virginianum*, showing 92 bivalent chromosomes.

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